100513-66-4; **6** (R_1 = $C_{17}H_{35}$), 100486-10-0; **7** (R_1 = $C_{13}H_{27}$), 100486-14-4; **7** (R_1 = $C_{15}H_{31}$), 100486-15-5; **7** (R_1 = $C_{17}H_{35}$), 100486-16-6; **8** (R = $C_{15}H_{31}$), 100486-19-9; **9** (R = $C_{15}H_{31}$), 100513-67-5; **10** (R_2 = $C_{13}H_{25}$), 28758-96-5; **10** (R_2 = $C_{15}H_{29}$), 28758-98-7; **10** (R_2 = $C_{17}H_{33}$), 28759-01-5; **14**, 100486-23-5; **15**, 50423-29-5; **16**, 100486-24-6; **17**, 100486-26-8; **19** (R_2 = $C_{13}H_{25}$), 100486-28-0; **19** (R_2 = $C_{15}H_{29}$), 100513-68-6; **19** (R_2 = $C_{17}H_{33}$), 100486-29-1; **20** (R_1 = $C_{13}H_{27}$), 100485-96-9; **20** (R_1 = $C_{15}H_{31}$), 65446-29-9; **20** (R_1 = $C_{17}H_{35}$), 100485-97-0; **21** (R_1 = $C_{13}H_{27}$), 100485-98-1; **21** (R_1 = $C_{15}H_{31}$), 38449-25-1; **21** (R_1 = $C_{17}H_{35}$), 100485-99-2; **22** (R_1 = $C_{13}H_{27}$), 100486-00-8; **22** (R_1 = $C_{15}H_{31}$), 100486-01-9; **22** (R_1 = $C_{17}H_{35}$), 100486-02-0; **23** (R_1 = $C_{13}H_{27}$), 100486-03-1; **23** (R_1 = $C_{15}H_{31}$), 100486-04-2; **23** (R_1 = $C_{17}H_{35}$), 100486-05-3; **24** (R_1 = $C_{13}H_{27}$), 100486-06-4; **24** (R_1 = $C_{15}H_{31}$), 100486-07-5; **24** (R_1 = $C_{17}H_{35}$), 100486-08-6; **25** (R_1 = $C_{13}H_{27}$), 100486-11-1; **25** (R_1 = $C_{15}H_{31}$), 100486-12-2; **25** (R_1 = $C_{17}H_{35}$), 100486-13-3; **26**, 100486-17-7; **28**, 68593-72-6; **29**, 100486-18-8; **32**, 100486-20-2; **33**, 100486-21-3; **34**, 100486-22-4; PDC, 492-89-7; PhOH, 108-95-2; $CH_3(CH_2)_{13}C(O)Cl$, 17746-08-6; $CH_3(CH_2)_{13}Br$, 112-71-0; *m*-$(MeO)_2C_6H_4$, 151-10-0; $CH_3(CH_2)_{14}Br$, 629-72-1; veratraldehyde, 120-14-9; resorcinol, 108-46-3; 2-*n*-pentadecylhydroquinone dimethyl ether, 100486-27-9.

Aminoglycoside Antibiotics. 6. Chemical Reactions of Aminoglycosides with Disodium Carbenicillin

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Aminoglycoside antibiotics including kanamycin A, tobramycin, and the gentamicin C complex reacted with 1 mol of disodium carbenicillin to give products derived from acylation of their amino groups by the β-lactam function of the carbenicillin. Amikacin was acylated by two carbenicillin units. Chromatographic analysis of fragments from the acid hydrolysis of these derivatives showed that the preferred site of acylation was in the 2-deoxystreptamine unit of the aminoglycosides. The two sites of acylation in amikacin were the 6′-amino group and the amino group in the aminohydroxybutyryl substituent. The derivatives had almost no antibacterial activity, and they were not toxic.

Combinations of aminoglycoside antibiotics with penicillins or cephalosporins have been shown to exert synergistic effects in killing bacteria. Such combinations are used clinically in the treatment of infections by Gram-negative rods, especially those caused by *Pseudomonas aeruginosa*.[1-4] However, in vitro studies have shown that high concentrations of penicillins such as carbenicillin and ticarcillin inactivate the aminoglycosides. This inactivation does not usually cause clinical problems, but it can be a serious consideration in patients with renal failure.[5-8]

A number of studies have been addressed to the relative reactivity of various β-lactams and aminoglycosides with each other.[9-11] From them, it appears that penicillins are more reactive than cephalosporins.[12] Carbenicillin most readily produced inactivation, followed in decreasing order by ticarcillin, benzylpenicillin, and ampicillin.[10,13] Among the aminoglycosides, tobramycin was the most readily inactivated, followed by gentamicin and kanamycin.[14,15] Netilmicin and amikacin were relatively resistant.[16,17] Most of these studies were made with blood serum as the vehicle.

Despite the high interest in the biological consequences of the penicillin–aminoglycoside interactions and the development of assay methods to detect the extent to which they have occurred in mixtures of these two types of antibiotics, little is known about the chemistry of the interaction. Weinstein and co-workers suggested that it involves a nucleophilic opening of the β-lactam ring by an amino group of the aminoglycoside with formation of an inactive amide (Figure 1).[18] This is a most reasonable explanation, but it has not been verified. Furthermore, it does not address the question of which amino group or groups are acylated. Aminoglycosides can have four or five amino groups, and it would be valuable to know if there is selectivity in their acylation. One study has been directed toward elucidation of the chemistry.[19] It involved the reaction of benzylpenicillin with kanamycin A and reported that they formed *O*-(benzylpenicilloyl)kanamycin, which was converted into *N*-(benzylpenicilloyl)kanamycin and benzylpenicilloic acid. We felt that this rather complex process merited further study with emphasis on stoichiometry and selectivity of the reaction.

On the basis of the foregoing considerations, we decided to examine the chemical interactions between disodium

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Table I. Preparation and Properties of Disodium Carbenicillin Derivatives of Aminoglycosides

aminoglycoside	equiv disodium carbenicillin	time, days	yield, %	dec temp, °C	$[\alpha]^{26}_{546}$, deg[a]	formula	anal.
kanamycin A	2.8	7	82	>250	+102.1	$C_{35}H_{54}N_6O_{17}SNa_2 \cdot H_2SO_4$	C, H, S[b]
tobramycin	2.8	7	71		+66.8	$C_{35}H_{53}N_7O_{15}SNa_2 \cdot 2H_2SO_4$	C, H, N[c]
gentamicin	3.7	15	57		+62.5	mixture	
amikacin	2.8	3	94	230–233	+89.4	$C_{56}H_{75}N_9O_{25}S_2Na_4 \cdot H_2SO_4$	C, S[d]

[a] All samples were 1 mg/100 mL in water. [b] N: calcd, 8.21; found, 7.29. [c] S: calcd, 8.84; found, 7.86. [d] H: calcd, 5.08; found, 5.68. N: calcd, 8.25; found, 7.56.

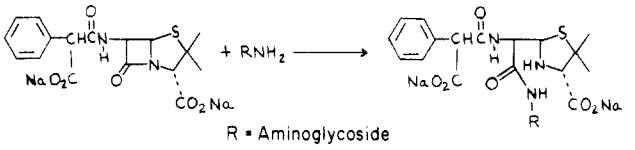

Figure 1.

carbenicillin and a variety of aminoglycosides including kanamycin A, amikacin, tobramycin, and the gentamicin C complex. All of these aminoglycosides, except kanamycin A, are used in combination with penicillins. Carbenicillin was chosen because of its putative high reactivity[13,17] and its clinical importance.

Chemistry. Solutions of the aminoglycoside sulfates in distilled water were treated at 25 °C with 2.7–4.0 mol of disodium carbenicillin, added in portions. When thin-layer chromatography (TLC) showed no remaining aminoglycoside (3–15 days), the solutions were concentrated under reduced pressure and the residues were treated with ethanol. Unreacted carbenicillin and its decomposition products were removed by washing with ethanol. The residual solids from kanamycin A or amikacin showed a single spot on TLC, whereas those from tobramycin or the gentamicin C complex showed two spots. There probably was overlap in the latter case because at least three products are expected. The solids were pure enough for combustion analysis, which showed 1:1 stoichiometry in all cases except that of amikacin (Table I). The formula for the amikacin product showed that it contained 2 mol of disodium carbenicillin per amikacin. The gentamicin product was not submitted to combustion analysis because of its derivation from a mixture of three components. The infrared spectrum of each product showed the absence of a β-lactam ring. No *O*-acyl group was present, but absorptions characteristic of amide and carboxylate groups were apparent (Experimental Section). Phenyl and methyl groups from the carbenicillin residue were apparent in the proton NMR spectrum. These data confirm the postulated structures shown in Figure 1, without indicating the positions of acylation of the aminoglycosides.

The sites of reaction on the aminoglycosides were determined by comparing the acid hydrolysis products of the starting aminoglycosides with those of their carbenicillin derivatives (Table II). The absence of an amino sugar or 2-deoxystreptamine unit in the hydrolysate indicates acylation of that unit by carbenicillin. This analysis assumes that hydrolysis of the amide bond does not occur. Evidence from the hydrolysates supports this assumption. Acid hydrolysis of aminoglycosides with 2′-amino groups [tobramycin, the gentamicins C, kanamycin B (included as a reference compound)] required more drastic conditions than those with 2′-hydroxyl groups.[20–22] Kanosamine (3-amino-3-deoxy-D-glucose; Figure 2) was found in the hydrolysates from the carbenicillin derivatives of kanamycin A, tobramycin, and amikacin (Table II), indicating that no acylation occurred in this unit. The corresponding garosamine unit (Figure 2) was found in the hydrolysate of the carbenicillin derivative of the gentamicin C complex. The hydrolysate from the kanamycin A derivative gave 6-amino-6-deoxy-D-glucose, and that from the mixture of

Table II. Hydrolysis Products of Aminoglycosides and Their Carbenicillin Products

compd	hydrolysis products
kanamycin B	0.32 (kanosamine); 0.16 (2,6-DDG);[a] 0.14 (2-deoxystreptamine)
kanamycin A	0.32 (kanosamine); 0.24 (6-ADG);[b] 0.14 (2-deoxystreptamine)
kanamycin A/carbenicillin	0.32 (kanosamine); 0.24 (6-ADG)[b]
tobramycin	0.32 (kanosamine); 0.23 (tobramine); 0.14 (2-deoxystreptamine)
tobramycin/carbenicillin	0.32 (kanosamine); 0.23 (tobramine); 0.14 (faint, 2-deoxystreptamine)
amikacin	0.32 (kanosamine); 0.24 (6-ADG);[b] 0.21 ((aminohydroxybutyryl)-2-deoxystreptamine)
amikacin/carbenicillin	0.32 (kanosamine)
gentamicin	0.53, 0.49, 0.38, 0.21 (purpurosamines A, C, and B and garosamine); 0.14 (2-deoxystreptamine)
gentamicin/carbenicillin	0.53, 0.49, 0.38, 0.21 (purpurosamines A, C, and B and garosamine)

[a] 2,6-Dideoxy-2,6-diamino-D-glucose. [b] 6-Amino-6-deoxy-D-glucose.

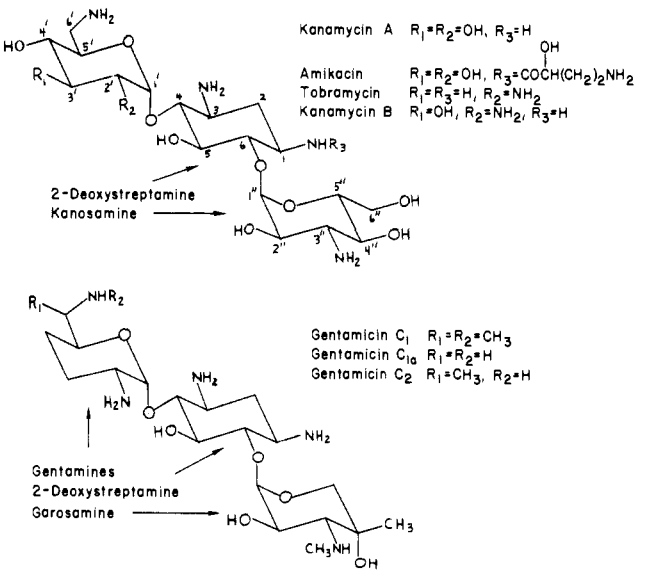

Figure 2.

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Table III. Antibacterial Activities of Aminoglycosides and Their Carbenicillin Derivatives[a]

organism[b]		carbenicillin	kanamycin	MIC, μg/mL tobramycin	gentamicins	kanamycin/carbenicillin	tobramycin/carbenicillin	gentamicin/carbenicillin
S. au	A-9537	1	0.05	0.03	0.06	>250	63	16
	A22210	>125	>125	8	32	>250	>250	>250
	A21978	>125	>125	32	0.25	>250	>250	>250
	A20240	8	>125	0.13	0.13	>250	>250	>250
	A22058	4	125	32	0.13	>250	>250	32
	A22231	2	8	16	0.13	>250	63	16
E. co	A22356	4	2	0.25	0.5	>250	>250	250
	A20697	32	2	0.13	0.25	>250	>250	250
	A-9632	8	2	0.25	0.25	>250	>250	250
	A20665	8	>125	1	0.5	>250	>250	>250
	A20683	>125	>125	16	32	>250	>250	>250
	A20895	63	2	0.5	32	>250	>250	>250
	A22045	32	4	4	63	>250	>250	>250
	A21218	32	32	8	0.25	>250	>250	250
	A20732	>125	63	8	16	>250	>250	>250
E. cl	A-9656	32	1	0.25	0.5	>250	>250	>250
	A20364	>125	>125	0.5	0.5	>250	>250	250
	A21006	>125	>125	1	0.5	>250	>250	250
	A21136	>125	32	16	2	>250	>250	>250
K. pn	A20468	>125	0.5	0.06	0.25	>250	>250	125
P. mi	A-9900	0.5	2	0.5	1	>250	>250	250
P. re	A-9637	0.5	0.5	0.13	0.06	250	125	32
	A21207	16	2	32	8	>250	>250	>250
P. st	A21210	1	0.5	2	1	>250	>250	>250
	A20894	8	4	16	125	>250	>250	>250
S. ma	A20019	16	1	2	1	>250	250	>250
P. ae	A21508	>125	>125	0.06	0.5	>250	250	>250
	A-9834A	32	63	0.13	0.25	>250	250	>250
	A20601	125	32	0.13	>125	>250	>250	>250
	A20717	>125	32	0.13	4	>250	>250	>250
	A20897	>125	>125	125	>125	>250	>250	>250
	A20653	125	>125	0.13	0.5	>250	250	>250
	A20741	125	>125	>125	>125	>250	>250	>250
	A21294	>125	63	16	1	>250	>250	>250
	A22233	>125	>125	125	>125	>250	>250	>250
	A21509	>125	>125	4	16	>250	>250	>250

[a] Assays performed at Bristol-Myers Co., Syracuse, NY. For details of the procedure see: Misiek, M.; Pursiano, T. A.; Crast, L. B.; Leitner, F.; Price, K. E. *Antimicrob. Agents Chemother.* **1972**, *1*, 54. [b] Abbreviations for the microorganisms: *S. ua* = *Staphylococcus aureus*; *E. co* = *Escherichia coli*; *E. cl* = *Enterobacter cloacae*; *K. pn* = *Klebsiella Pneumonia*; *P. mi* = *Proteus mirabilis*; *P. re* = *Proteus rettgeri*; *P. st* = *Proteus stuartii*; *S. ma* = *Serratia marcesens*; *P. ae* = *Pseudomonas aeruginosa*.

the gentamicin C derivatives gave all three purpurosamines (Figure 2), which limits the site of acylation in these compounds to the 2-deoxystreptamine unit. This unit was absent in both of the hydrolysates. Hydrolysis of the derivative of tobramycin gave tobramine (2,6-diamino-2,3,6-trideoxy-D-glucose), but a smaller spot for 2-deoxystreptamine showed in the chromatogram. This observation is consistent with the two spots shown by the derivative, and it probably means that acylation of tobramycin was not specific, with most of the reaction occurring on the 2-deoxystreptamine unit and less occurring on the tobramine unit (possibly on its 6-amino group).

There are two possible sites, *N*-1 and *N*-3, for acylation in the 2-deoxystreptamine unit of kanamycin A, tobramycin, and the gentamicins. It should be possible to distinguish between them by a ^{13}C NMR study, on the basis of the reported chemical shifts for the β-carbon atoms that accompany acylation and the further shifts that occur when the spectra are determined at low pH.[23,24] We investigated this possibility for the carbenicillin derivative of kanamycin A. An initial difficulty was encountered in determining the ^{13}C NMR spectrum at pH 1 for comparison with literature values because the sample underwent decomposition. However, it was possible to take the spectrum in D_2O at pH 4. Protonation of all amines in the kanamycin A moiety should be complete at this acidity. Thus, the comparison is valid. Table IV shows the chemical shifts for C-6 (β to N-1) and C-4 (β to N-3) in neutral or mildly basic)[25] and acid solutions for amikacin (N-1 acylation), isoamikacin (N-3 acylation), and the carbenicillin derivative. It is not possible to assign a position of acylation from the values at higher pH because chemical shifts for the carbenicillin derivative are consistent with

Table IV. Chemical Shifts in the ^{13}C NMR Spectra of Acyl Derivatives of Kanamycin A at Neutral and Acid pH[a]

	chem shifts, ppm (from Me_4Si)				
	neutral		acid		
compd	C-6	C-4	C-6	C-4	
amikacin	81.2	87.6	81.2	79.8	1.4
isoamikacin	83.0	81.6	84.6	78.9	5.7
kanamycin A/carbenicillin	85.2	81.6	84.3	78.9	5.4
tobramycin/carbenicillin			84.1	76.1	8.0

[a] Amikacin and isoamikacin values from ref 23. Carbenicillin derivative of kanamycin A determined at pH 7.4 and pH 4.0 and carbenicillin derivative of tobramycin determined at pH 5.0, both in D_2O.

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(25) Literature references specify "free base" without giving the exact pH.

either amikacin or isoamikacin. However, a clear distinction can be made in acid solution because the chemical shift for C-4 changes more than that for C-6.[23,24] The values for the carbenicillin derivative are almost exactly the same as those for isoamikacin, which demonstrates that acylation by carbenicillin has occurred at N-3. This conclusion is consistent with studies on the chemical acetylation of aminoglycosides that show preferential reaction at N-3.[26]

The ^{13}C NMR spectrum of the carbenicillin derivative of tobramycin showed chemical shifts in acid that resembled those of isoamikacin and the carbenicillin derivative of kanamycin A, although the C-4 peak was shifted further upfield Table IV). We consider this evidence suggestive, but not conclusive, for N-3 acylation. Assignment of the acylation site on the gentamicin mixture was not attempted because of the complexity of the spectrum.

Amikacin is a special case because of the 4-amino-2-hydroxybutyryl substituent on N-1 of its 2-deoxystreptamine unit (Figure 2). Hydrolysis of amikacin gives kanosamine and 6-amino-6-deoxy-D-glucose, but no 2-deoxystreptamine is found because the substituent is stable. A spot for *N*-1-(4-amino-2-hydroxybutyryl)-2-deoxystreptamine is found instead. Hydrolysis of the bis(carbenicillin) derivative of amikacin gives only kanosamine, which shows that acylation occurred in the other two units. The amino group acylation in the N-1-substituted 2-deoxystreptamine unit must be the one in the 4-amino-2-hydroxybutyryl substituent because the ^{13}C NMR spectrum shows C-6 at 82.3 ppm and C-4 at 86.4 ppm. If N-3 had been acylated, a significant upfield shift in C-4 (to about 81 ppm) would have occurred, as is observed in isoamikacin.[23]

Biology. Samples of the urine from one patient receiving the tobramycin/carbenicillin combination and one receiving the gentamicin/carbenicillin combination were made available for this study.[27] Thin-layer chromatography showed in each case a spot identical in R_f value to that of our corresponding in vitro product. It is not known to what extent the products in the urine sample were formed before or after collection. Previous studies have shown that reactions occur during storage, even at 5 °C,[18] and we did not receive the samples immediately after collection. However, the relevant question is the identity of our synthetic samples with those found in a biological fluid, rather than the pharmacokinetics of product formation.

Samples of the disodium carbenicillin derivatives of kanamycin A, tobramycin, and the gentamicin C complex were tested at the Bristol-Myers Co. for antibacterial activity and toxicity. The antibacterial activities of these derivatives are compared with those of the parent aminoglycosides in Table III. This table shows than none of the derivatives have useful antibacterial activity. However, the derivatives of tobramycin and the gentamicin C complex are not completely inactive. An important finding is that none of these derivatives were lethal to mice upon intravenous administration of a 1000 mg/kg dose. In contrast, kanamycin A was lethal at 500 mg/kg, and tobramycin or the gentamicin C complex was lethal at 200 mg/kg. Thus, although the reaction of aminoglycosides with disodium carbenicillin might result in decreased antibacterial activity in patients with renal failure, it does not appear to add the hazard of toxic products.

Experimental Section

Melting (decomposition) points were recorded on a Mel-Temp melting point apparatus and are uncorrected. IR spectra were taken on a Beckman IR-33 spectrometer with samples prepared as potassium bromide pellets. Absorptions are reported in reciprocal centimeters. NMR spectra were taken on a JEOL FX-90Q 90-MHz spectrometer, and absorptions are reported as downfield from Me_4Si. Optical rotations were determined on a Perkin-Elmer digital polarimeter. Elemental analyses were performed by the University of Arizona Analytical Center or Micanal, Inc., Tucson, AZ. Analytical results were within ±0.4% of theoretical values unless specified otherwise.

Reactions of Aminoglycosides with Disodium Carbenicillin. Solutions of the commercially available sulfate salts of kanamycin A, tobramycin, the gentamicin C complex and amikacin in distilled water at 25 °C were treated with disodium carbenicillin. In a typical experiment, 0.48 g (0.85 mmol) of tobramycin sulfate and 0.50 g (1.18 mmol) of disodium carbenicillin in 40 mL of water were kept for 3 days, after which another 0.50 g of disodium carbenicillin was added. After 7 days, TLC showed no remaining tobramycin and two new spots [$CHCl_3$/CH_3OH/28% NH_4OH (2:2:1) on silica gel with ninhydrin spray]. The mixture was concentrated under reduced pressure to a small volume and treated with ethanol, whereupon white solid precipitated. It was washed thoroughly with ethanol and dried under vacuum. The IR spectrum showed no absorption for β-lactam or *O*-acyl groups, but amide bands were present at 1650, 1600, and 1525 cm^{-1} and carboxylate anion was at 1370 cm^{-1}. The NMR spectrum showed phenyl (δ 7.35) and methyl (δ 1.45) groups as well as typical absorptions for aminoglycosides.

Table I gives the molar ratios of reactants and times for the disappearance of each aminoglycoside. It also lists the product yield, decomposition point, optical rotation, and elemental analyses.

Hydrolysis of Aminoglycosides and Their Carbenicillin Derivatives. The reaction conditions depended upon the particular aminoglycoside. Kanamycin A, amikacin, and their carbenicillin derivatives (10-mg samples) were heated with 4 N HCl for 15 min on a steam bath, whereas tobramycin, gentamicin and their derivatives, kanamycin B were heated with 6 N HCl for 30 min. The resulting solutions were cooled and concentrated under reduced pressure, the residues were dissolved in 0.5 mL of water, and these solutions were analyzed by TLC on precoated silica gel plates using the solvent system $CHCl_3/CH_3OH/17\%$ NH_4OH (2:2:1). After development, the plates were sprayed with ninhydrin reagent and warmed. Table II lists the R_f values of the units obtained from the hydrolyses.

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